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PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re:	Patent Application of Richard A. Fishel et al.	: Group Art Unit: Not Yet Assigned
Appln. No.:	Not Yet Assigned	: Examiner: : Not Yet Assigned
Filed:	Herewith	:
For:	COMPOSITIONS, KITS, AND METHODS FOR EFFECTING ADENINE NUCLEOTIDE MODULATION OF DNA MISMATCH RECOGNITION PROTEINS	: Attorney Docket : No. **9855-6U3** : **(OTT-3026-2)**

PRELIMINARY AMENDMENT

Prior to calculation of the filing fee for the application referenced above, please amend the application as follows.

In the Specification:

Please delete the paragraph designated "**[0047]**" that appears at pages 14 and 15 of the specification, and substitute the following paragraph in place thereof.

[0047] -- Figure 1, comprising Figures 1A, 1B, 1C, 1D, 1E, and 1F, depict binding of hMSH2:hMSH6 heterodimer to mismatched and non-mismatched duplex DNA. Figure 1A is an image of the results of a gel mobility shift assay performed using the G/T-mismatched 81-base pair duplex DNA substrate described herein. The concentrations of heterodimer used in the assay were, in nanomolar, 0 in A, 6 in B, 19 in C, 32 in D, 64 in E, 97 in F, 129 in G, 161 in H, 193 in I, 257 in J, and 322 in K. The position of the S-shifted electrophoretic band is indicated by "S". Figure 1E is a graph which depicts the relationship between the concentration of heterodimer and the amount of product corresponding to the S-shifted electrophoretic band in Figure 1A, as assessed using a phosphoimaging device. Figure 1B is an image of the results of a

gel mobility shift assay performed using the homologous 81-base pair duplex DNA substrate described herein. The concentrations of heterodimer used in the assay were, in nanomolar, 0 in A, 6 in B, 19 in C, 32 in D, 64 in E, 129 in F, 225 in G, 322 in H, 386 in I, and 482 in J. The position of the NS-shifted electrophoretic band is indicated by "NS". Figure 1F is a graph which depicts the relationship between the concentration of heterodimer and the amount of product corresponding to the NS-shifted electrophoretic band in Figure 1B, as assessed using a phosphoimaging device. Figure 1C is an image which depicts the results of a DNase footprint assay performed using the 81-base pair G/T-mismatched duplex DNA substrate described herein. The concentrations of 81-base pair were, in nanomolar, 0 in A, 13 in B, 32 in C, and 97 in D. The position of the G residue of the G/T-mismatched substrate is indicated by "G", and the approximate region of the substrate protected from DNase cleavage by the heterodimer is indicated by a vertical line. Figure 1D is an image which depicts the results of a DNase footprint assay performed using the homologous 81-base pair duplex DNA substrate described herein. The concentrations of heterodimer used in the assay were, in nanomolar, 0 in A, 161 in B, 322 in C, and 482 in D. The position of the G/C base pair corresponding to the G/T-mismatched base pair of the mismatched substrate is indicated by "G". --

Please delete the paragraph designated "**[0048]**" that appears at pages 15 and 16 of the specification, and substitute the following paragraph in place thereof.

[0048] -- Figure 2, comprising Figures 2A, 2B, 2C, and 2D, depicts the results of gel mobility shift assays used to assess the ability of various adenine nucleotides to dissociate MSH dimer from the mismatch site, corresponding to the S-shifted electrophoretic band, such that the MSH dimer, corresponding to the NS-shifted electrophoretic band, exhibited DNA-associated diffusion. Figure 2A is an image of an assay in which the product corresponding to the S-shifted electrophoretic band was incubated in the presence of ATP at the following concentration, in micromolar, 0 in A and B, 0.5 in C, 1 in D, 2 in E, 3.9 in F, 7.8 in G, 15.6 in H, 31.3 in I, 62.5 in J, and 125 in K. Figure 2B is an image of an assay in which the product corresponding to the S-shifted electrophoretic band was incubated in the presence of adenosine-5'-O-3'-thiotriphosphate

(ATP-γ-S) at the following concentration, in micromolar, 0 in A and B, 0.5 in C, 1 in D, 2 in E, 3.9 in F, 7.8 in G, 15.6 in H, 31.3 in I, 62.5 in J, and 125 in K. Figure 2C is an image of an assay in which the product corresponding to the S-shifted electrophoretic band was incubated in the presence of ADP at the following concentration, in micromolar, 0 in A and B, 25 in C, and 100 in D. In lane A of each of Figures 2A, 2B, and 2C, no heterodimer was included in the assay mixture. Figure 2D is a graph which depicts quantitated results obtained using the results depicted in Figures 2A, 2B, and 2C, as assessed using a phosphoimaging device. --

Please delete the paragraph designated "**[0052]**" that appears at pages 16 and 17 of the specification, and substitute the following paragraph in place thereof.

[0052] -- Figure 6, comprising Figures 6A, 6B, 6C, and 6D, depicts the results of experiments performed to assess the effects of ATP, homologous DNA, or both, on the dissociation of the hMSH2:hMSH6 heterodimer from DNA. Figure 6A is an image of the results obtained from gel mobility shift assays in which heterodimer-bound mismatched DNA was incubated with ATP for the following time, in minutes, 0 in A and B, 0.5 in C, 1 in D, 2 in E, 3 in F, 4 in G, 5 in H, 7.5 in I, and 10 in J. Figure 6B is an image of the results obtained from gel mobility shift assays in which heterodimer-bound mismatched DNA was incubated with ATP and a 400-fold excess of homologous DNA for the following time, in minutes, 0 in A and B, 0.5 in C, 1 in D, 2 in E, 3 in F, 4 in G, 5 in H, 7.5 in I, and 10 in J. Figure 6C is an image of the results obtained from gel mobility shift assays in which heterodimer-bound mismatched DNA was incubated with a 400-fold excess of homologous DNA for the following time, in minutes, 0 in C, 5 in D, and 10 in E. Figure 6D is an image of the results obtained from gel mobility shift assays in which the heterodimer was incubated with homoduplex DNA probe for fifteen minutes at 37°C (Lane B), the assay mixture was cooled to 4°C, and a 1,100-fold excess of unlabeled competitor homoduplex DNA was added (Lane C). In each of Figure 6A, 6B, 6C, and 6D, the assay mixtures corresponding to lane A did not comprise the heterodimer. --

Please delete the paragraph designated "**[0054]**" that appears at page 17 of the specification, and substitute the following paragraph in place thereof.

[0054] -- Figure 8, comprising Figures 8A, 8B, 8C, and 8D, lists the nucleotide sequence of single nucleotide chains of some of the 39- and 81-base pair DNA substrates described herein. Figure 8A lists SEQ ID NO: 2. Figure 8B lists SEQ ID NO: 3. Figure 8C lists SEQ ID NO: 5. Figure 8D lists SEQ ID NO: 6. --

Please delete the paragraph designated "**[0064]**" that appears at page 20 of the specification, and substitute the following paragraph in place thereof.

[0064] -- Figure 18 is a diagram which illustrates a model of hMSH2 consensus interaction with hMSH3 or hMSH6. The interaction regions of hMSH2, hMSH3, and hMSH6 are indicated are connected with lines that illustrate the specificity of each region to its corresponding interaction partner region. The nucleotide binding regions of hMSH2, hMSH3, and hMSH6 are indicated. The location of HNPCC-associated mutations tested in these studies are illustrated as black diamonds. --

Please delete the paragraph designated "**[0065]**" that appears at page 20 of the specification, and substitute the following paragraph in place thereof.

[0065] -- Figure 19, comprising Figures 19A through 19G, lists the nucleotide sequence of cDNA encoding hMSH5 (SEQ ID NO: 30) and the putative amino acid sequence of hMSH5 (SEQ ID NO: 29). --

Please delete the paragraph designated "**[0101]**" that appears at page 29 of the specification, and substitute the following paragraph in place thereof.

-- The invention relates to a method of modifying a mismatched duplex DNA. The method comprises contacting a MutS homolog (MSH) dimer and the mismatched duplex DNA in the presence of a binding solution. The binding solution comprises either ADP and ATP, and the concentration of ATP in the binding solution is less than 5 micromolar, less than

about 3 micromolar, 1 micromolar or less, preferably less than about 0.3 micromolar, and more preferably wherein the binding solution is substantially free of ATP. Alternately, ADP is used in the absence of ATP, or at least in excess with respect to ATP (i.e. ADP at a 2-fold, 10-fold, or 100-fold or greater excess relative to ATP). The MSH dimer thereby binds ADP. When the ADP-bound MSH dimer is contacted with the mismatched duplex DNA, the dimer associates with the mismatched region of the DNA, thus forming a modified mismatched duplex DNA. --

A marked-up copy of each of the replacement paragraphs is enclosed in the document entitled "**Marked-Up Copy of Replacement Specification Paragraphs**".

In the Claims:

Please cancel claims 1-31, 34, 36, 38, 41, 43, and 55, without prejudice to inclusion of the subject matter of these claims in one or more additional patent applications.

Please amend claim 48 to read as follows.

48. (Amended) A cell line which is nullizygous for both Msh2 and p53, does not express Msh2 or p53, and exhibits a phenotype selected from the group consisting of a predisposition for carcinogenesis and a predisposition for apoptosis, wherein said cell line is made by culturing a cell obtained from the nonhuman mammal of claim 44.

A marked-up copy of claim 48, as amended herein, is enclosed in the document designated "**Marked-Up Copy of Claim 48**".

REMARKS

Claims 35, 37, 39, 40, 42, and 44-54 are pending in the present application. Claims 1-31, 34, 36, 38, 41, 43, and 55 have been canceled. Claims 35, 37, 39, 40, 42, 44-47, and 49-54 is an independent claim. Claims 48 is a dependent claim, and has been amended to depend from claim 44.

Support in the Specification

Paragraphs 47, 48, 52, 54, 64, and 65 of the specification have been amended to move information from the Figures filed with the parent non-provisional application to the corresponding figure legend or otherwise conform the figure legends to the changes made to the drawings in order to comply with formal drawing requirements.

Paragraph 101 of the specification has been amended by adding specific ATP concentrations recited in the priority application (see page 20, lines 23-26 of U.S. provisional patent application 60/066,977).

For the foregoing reasons, the Applicants respectfully contend that none of the amendments made herein includes new matter.

Reason for Filing Declaration of Gary D. Colby Pursuant to 37 C.F.R. § 1.312

During prosecution of the parent non-provisional application, an issue was raised regarding whether the reference by Acharya et al. (1996, Proc. Natl. Acad. Sci. USA 93:13629-13634) can be cited as prior art. The Applicants respectfully reply that Acharya is not prior art.

A printed reference qualifies as prior art pursuant to 35 U.S.C. § 102(b) only if the reference has an effective publication date that is more than one year earlier than the effective filing date of an application to which the present application is entitled for priority and which describes the same or similar subject matter. The present application is entitled for priority to U.S. provisional patent application 60/066,977 ("the '977 application," filed on November 28, 1997).

The Acharya et al. reference has a nominal publication date of November 26, 1996. As set forth in the Declaration of Gary D. Colby Pursuant to 37 C.F.R. § 1.312 enclosed herewith, a representative of the publisher of the Acharya reference confirmed that the November 26, 1996 issue of PNAS (Volume 93, Number 24) was mailed on November 26, 1996. The Applicants therefore respectfully contend that this publication could not have been available to the public earlier than November 27, 1996 (i.e., the U.S. Postal Service will not

ordinarily deliver an item the same day it is mailed). The effective publication date of the Acharya et al. reference is therefore not earlier than November 27, 1996.

As explained at MPEP § 706.02(a),

> "*Where the last day of the year dated from the date of publication* [of a reference] *falls on a* [] *Federal holiday, the publication is not a statutory bar under 35 U.S.C. § 102(b) if the application was filed on the next succeeding business day.*"

November 27, 1997 was Thanksgiving Day, a Federal holiday in the District of Columbia. The '977 application was filed on the next succeeding business day, November 28, 1997. Therefore, the Applicants believe that the Acharya reference is not prior art pursuant to any subsection of 35 U.S.C. § 102, and that the Acharya reference cannot be cited by the Examiner as prior art.

Summary

The Applicants respectfully contend that each of claims 35, 37, 39, 40, 42, and 44-54 is in condition for allowance. Early and favorable consideration of these claims is respectfully requested.

Respectfully submitted,

Richard Fishel *et al.*

22 August 2001 By: _____
(Date)

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Enclosures: Marked-Up Copy of Replacement Specification Paragraphs
Marked-Up Copy of Claim 48

Marked-Up Copy of Replacement Specification Paragraphs, as Amended in the Preliminary Amendment Filed Herewith

Paragraph **[0047]** *has been amended as follows.*

[0047] Figure 1, comprising Figures 1A, 1B, 1C, 1D, 1E, and 1F, depict binding of hMSH2:hMSH6 heterodimer to mismatched and non-mismatched duplex DNA. Figure 1A is an image of the results of a gel mobility shift assay performed using the G/T-mismatched 81-base pair duplex DNA substrate described herein. The concentrations of heterodimer used in the assay ~~are indicated along the top of the image~~ were, in nanomolar, 0 in A, 6 in B, 19 in C, 32 in D, 64 in E, 97 in F, 129 in G, 161 in H, 193 in I, 257 in J, and 322 in K. The position of the S-shifted electrophoretic band is indicated by "S". Figure 1E is a graph which depicts the relationship between the concentration of heterodimer and the amount of product corresponding to the S-shifted electrophoretic band in Figure 1A, as assessed using a phosphoimaging device. Figure 1B is an image of the results of a gel mobility shift assay performed using the homologous 81-base pair duplex DNA substrate described herein. The concentrations of heterodimer used in the assay ~~are indicated along the top of the image~~ were, in nanomolar, 0 in A, 6 in B, 19 in C, 32 in D, 64 in E, 129 in F, 225 in G, 322 in H, 386 in I, and 482 in J. The position of the NS-shifted electrophoretic band is indicated by "NS". Figure 1F is a graph which depicts the relationship between the concentration of heterodimer and the amount of product corresponding to the NS-shifted electrophoretic band in Figure 1B, as assessed using a phosphoimaging device. Figure 1C is an image which depicts the results of a DNase footprint assay performed using the 81-base pair G/T-mismatched duplex DNA substrate described herein. The concentrations of 81-base pair ~~are indicated along the top of the image~~ were, in nanomolar, 0 in A, 13 in B, 32 in C, and 97 in D. The position of the G residue of the G/T-mismatched substrate is indicated by "G", and the approximate region of the substrate protected from DNase cleavage by the heterodimer is indicated by a vertical line. Figure 1D is an image which depicts the results of a DNase footprint assay performed using the homologous 81-base pair duplex DNA substrate described herein. The concentrations of heterodimer used in the assay ~~are indicated along the top of the image~~ were, in nanomolar, 0 in A, 161 in B, 322 in C, and 482 in

D. The position of the G/C base pair corresponding to the G/T-mismatched base pair of the mismatched substrate is indicated by "G".

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Paragraph **[0052]** *has been amended as follows.*

[0052] Figure 6, comprising Figures 6A, 6B, 6C, and 6D, depicts the results of experiments performed to assess the effects of ATP, homologous DNA, or both, on the dissociation of the hMSH2:hMSH6 heterodimer from DNA. Figure 6A is an image of the results obtained from gel mobility shift assays in which heterodimer-bound mismatched DNA was incubated with ATP for the following time ~~indicated in the image~~, in minutes, 0 in A and B, 0.5 in C, 1 in D, 2 in E, 3 in

F, 4 in G, 5 in H, 7.5 in I, and 10 in J. Figure 6B is an image of the results obtained from gel mobility shift assays in which heterodimer-bound mismatched DNA was incubated with ATP and a 400-fold excess of homologous DNA for the following time ~~indicated in the image~~, in minutes, 0 in A and B, 0.5 in C, 1 in D, 2 in E, 3 in F, 4 in G, 5 in H, 7.5 in I, and 10 in J. Figure 6C is an image of the results obtained from gel mobility shift assays in which heterodimer-bound mismatched DNA was incubated with a 400-fold excess of homologous DNA for the following time ~~indicated in the image~~, in minutes, 0 in C, 5 in D, and 10 in E. Figure 6D is an image of the results obtained from gel mobility shift assays in which the heterodimer was incubated with homoduplex DNA probe for fifteen minutes at 37°C (Lane ~~A~~ B), the assay mixture was cooled to 4°C, and a 1,100-fold excess of unlabeled competitor homoduplex DNA was added (Lane ~~B~~ C). In each of Figure 6A, 6B, 6C, and 6D, " " ~~indicates~~ the assay mixtures ~~which~~ corresponding to lane A did not comprise the heterodimer.

Paragraph **[0054]** *has been amended as follows.*

[0054] Figure 8, comprising Figures 8A, 8B, 8C, and 8D, lists the nucleotide sequence of single nucleotide chains of some of the 39- and 81-base pair DNA substrates described herein ~~(SEQ ID NOS: 2, 3, 5, and 6)~~. Figure 8A lists SEQ ID NO: 2. Figure 8B lists SEQ ID NO: 3. Figure 8C lists SEQ ID NO: 5. Figure 8D lists SEQ ID NO: 6.

Paragraph **[0064]** *has been amended as follows.*

[0064] Figure 18 is a diagram which illustrates a model of hMSH2 consensus interaction with hMSH3 or hMSH6. The interaction regions of hMSH2, hMSH3, and hMSH6 are indicated ~~in gray and~~ are connected with lines that illustrate the specificity of each region to its corresponding interaction partner region. The nucleotide binding regions of hMSH2, hMSH3, and hMSH6 are indicated ~~as black boxes~~. The location of HNPCC-associated mutations tested in these studies are illustrated as black diamonds.

Paragraph **[0065]** *has been amended as follows.*

[0065] Figure 19, comprising Figures 19A~~, 19B, and 19C~~ through 19G, lists the nucleotide sequence of cDNA encoding hMSH5 (SEQ ID NO: 30) and the putative amino acid sequence of hMSH5 (SEQ ID NO: 29).

Paragraph **[0101]** *has been amended as follows.*

[0101] The invention relates to a method of modifying a mismatched duplex DNA. The method comprises contacting a MutS homolog (MSH) dimer and the mismatched duplex DNA in the presence of a binding solution. The binding solution comprises either ADP and ATP, and the concentration of ATP in the binding solution is less than 5 micromolar, less than about 3 micromolar, 1 micromolar or less, preferably less than about 0.3 micromolar, and more preferably wherein the binding solution is substantially free of ATP. Alternately, ADP is used in the absence of ATP, or at least in excess with respect to ATP (i.e. ADP at a 2-fold, 10-fold, or 100-fold or greater excess relative to ATP). The MSH dimer thereby binds ADP. When the ADP-bound MSH dimer is contacted with the mismatched duplex DNA, the dimer associates with the mismatched region of the DNA, thus forming a modified mismatched duplex DNA.

Marked-Up Copy of Claim 48

48. (Amended) A cell line which is nullizygous for both Msh2 and p53, does not express Msh2 or p53, and exhibits a phenotype selected from the group consisting of a predisposition for carcinogenesis and a predisposition for apoptosis, wherein said cell line is made by culturing a cell obtained from the nonhuman mammal of claim ~~56~~ 44.